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The Effects of Campath 1H upon Graft-Versus-Host Disease, Infection, Relapse, and Immune Reconstitution in Recipients of Pediatric Unrelated Transplants

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ABSTRACT

Graft-versus-host disease (GVHD) is a cause of serious morbidity and mortality in >50% of recipients of unrelated hematopoietic stem cell transplantation (HSCT). We performed a trial using Campath 1 H pre- and post-HSCT in an attempt to decrease the incidence of GVHD without increasing the risk of infection or relapse. Patients were retrospectively compared to a population of patients who received antithymocyte globulin (ATG) pre- and post-HSCT. Twenty-seven patients were evaluated for this study. Fourteen patients received Campath 1H and 13 patients received ATG. Demographics of patients who received Campath 1H consisted of 9 males and 5 females, with a median age of 13 years (3-17.8 years). Thirteen patients received unrelated bone marrow and 1 patient received unrelated PBSC. Demographics of patients receiving ATG consisted of 9 males, 4 females with a median age of 7.4 years (21 months-19 years). Twelve patients received unrelated bone marrow and 1 patient received unrelated PBSC. Diagnoses were similar between the 2 groups. Patients who received Campath1H received a total dose of 52 mg/m^2 pre-HSCT and 20 mg/m^2 post-HSCT. Patients who received ATG received a total dose of 60 mg/kg pre-HSCT and 100 mg/kg post-HSCT. GVHD prophylaxis and supportive care measures were similar in both groups, including aggressive antimicrobial therapy. There was a significant difference in the incidence of severe (grade III and grade IV) GVHD between the 2 groups (Campath [0 of 14] versus ATG [6 of 13], $P = .006$). Among the patients who were transplanted for leukemia, there was no significant difference between the 2 groups in terms of relapse (Campath [2 of 14] versus ATG [4 of 9], $P = 0.16$). The 100-day survival between the 2 groups was not significantly different. Patients receiving Campath 1H had the presence of CD3$^+$ T cells (>30 cells/mL) in their peripheral blood later than in those who received ATG (64.5 days [Campath 1H] versus 27days [ATG], $P = .001$). The median time to the development of a normal PHA response occurred later in the Campath 1H arm (283 days[(Campath 1H] versus 88 days [ATG], $P = .0001$). The median time to an antigen specific response also occurred later in those receiving Campath 1H (365 days [Campath 1H] versus 150 days [ATG], $P = .004$). There was no significant difference between the 2 groups in terms of fungal or viral infections. Campath 1H is effective in decreasing the incidence of GVHD without increasing the risk of relapse. Although there is a significant delay in immune reconstitution, there was no increase in infectious complications or relapse in recipients of Campath 1H. Further studies are warranted to assess if a lack of difference in infection rates are still demonstrated in larger cohorts.
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KEY WORDS

Campath 1H • Graft-versus-host disease • Hematopoietic stem cell transplantation • Immune reconstitution

INTRODUCTION

Graft-versus-host disease (GVHD) represents one of the major causes of morbidity and mortality among patients receiving a matched-unrelated or mismatched-related hematopoietic stem cell transplant (HSCT). At Childrens Hospital Los Angeles (CHLA), antithymocyte globulin (ATG) has been used as a standard part of our conditioning and post-HSCT prophylaxis of GVHD for patients receiving a matched-unrelated HSCT (MUD) and a mismatched-related HSCT. Pre-HSCT ATG has been used to deplete the host's immune system to reduce the risk of rejection. Post-HSCT ATG has been used to decrease the donor T cells in vivo to reduce the risk of GVHD. Despite these measures, a majority of our MUD and mismatched-related patients develop clinically significant GVHD.

Alemtuzumab (Campath 1H) is a humanized monoclonal antibody to CD52, an antigen expressed by T and B lymphocytes, eosinophils, monocytes, natural killer cells, and some antigen-presenting dendritic cells [1]. The majority of $CD34^+$ stem cells and granulocytes are CD52 negative, and are therefore only modestly affected by the use of Campath 1H [2,3]. Campath 1H given prior to transplantation remains present after the graft infusion and provides an in vivo T cell depletion resulting in a lower risk of acute GVHD a(GVHD) [4-6]. However, there has been a high incidence of adenoviral infections [7,8] and CMV reactivation [9,10] associated with the use of Campath 1H. Theoretically, in vivo T cell depletion of donor grafts could lead to increased rates of leukemia relapse, from abrogation of the graft-versus-leukemia effect.

We replaced ATG with Campath 1H in patients receiving unrelated HSCT and mismatched-related HSCT to determine if we could decrease the incidence of GVHD, without increasing the incidence of viral and fungal infections or relapse.

PATIENTS AND METHODS

All patients who were scheduled to receive a matched- or mismatched-unrelated or a mismatched-related bone marrow or peripheral blood HSCT between April 2004 and June 2006 at CHLA, were eligible for this protocol. This study of the comparison of Campath 1H versus ATG was approved by the Childrens Hospital Committee on Clinical Investigations (institutional review board). Consent was obtained from the parents or the legal guardians and the subjects >18 years of age. Assent was obtained from subjects >7 years and <18 years of age. Patients who received ATG for their GVHD prophylaxis between January 2000 and March 2004 were used as historic controls. Patients who received an unrelated umbilical cord blood transplant were treated on a different protocol and were not eligible for this study.

Patient characteristics of study patients are shown in Table 1 and donor characteristics are shown in Table 2.

Table 1. *Patient Characteristics*

	ATG (n = 13)	Campath 1H (n = 14)
Males:Females	9:4	9:5
Median age at time of HSCT	7.4 years (3.4-19 years)	13 years (3-17.8 years)
Diagnosis		
ALL		
(Induction failure)	1	1
(CR2)	2	2
(CR3)	0	1
AML		
(Induction failure)	0	1
(CR2)	3	6
CML (total)		
(CP1)	2	3
(CP2)	1	0
SAA	2	0
ALD	1	0
CID	1	0
Conditioning regimen		
Busulfan/cytoxan	7	8
TBI/etoposide	4	6
Procarabazine/Cy/TLI	2	0
Median number of total nucleated cells (TNC) infused/kg recipient body weight	5×10^8 TNC/kg (1.4×10^8-11.6×10^8)	4.8×10^8 TNC/kg (2.3×10^8-38.6×10^8)

HSCT indicates hematopoietic stem cell transplantation; ATG, antithymocyte globulin; ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; SAA, severe aplastic anemia; ALD, adrenoleukodystrophy; CID, combined immunodeficiency; TBI, total body irradiation; TLI, total lymphoid irradiation; TNC, total nucleated cells.

Table 2. *Donor Characteristics*

	ATG (n = 13)	Campath 1H (n = 14)
Bone marrow/peripheral blood	12/1	13/1
HLA antigen mismatch		
0	12	10
1	1	3
2	0	1
Donor type		
Mismatched related	1	1
Matched unrelated	12	13
Sex match		
Male to male	5	7
Male to female	1	3
Female to male	3	3
Female to female	2	1

ATG indicates antithymocyte globulin.

Conditioning Regimens and GVHD Prophylaxis

All patients received a myeloablative conditioning regimen. The schematic diagram for the Campath 1H patients is seen in Figure 1. Campath 1H patients received a total of 52 mg/m^2 divided over 3 days pre-HSCT (days −6, −4, and −2) and 2 doses post-HSCT of 10 mg/m^2/dose on days 1 and 2. ATG recipients were given a regimen of ATGAM 20 mg/kg/dose for 4 doses pre-HSCT (days −7, −5, −3, and −1) and 20 mg/kg/dose × 5 doses post-HSCT (days 5, 7, 9, 11, and 13). All patients on both the Campath 1H and ATG arms received short course of methotrexate (10 mg/m^2) post-HSCT on days 3, 6, 11, and 18. Some patients with transplant-related toxicity (ie, venoocclusive disease (VOD) or severe mucositis) did not receive the 4th dose of MTX. All patients also received tacrolimus post-HSCT for GVHD prophylaxis. If patients were unable to tolerate tacrolimus, then they were changed to Cyclosporin A. aGVHD was graded according to the Glucksberg scale [11].

Campath Levels

The basis for the determination of free Campath1H levels is based on the specific binding of Campath 1H to normal human peripheral blood CD3$^+$ lymphocytes. The concentration of free anti-CD52 antibody in patient samples was determined by incubating heat-inactivated patient serum with peripheral blood leukocytes from normal donors, followed by detection of Campath1H bound to the cells using FITC-labeled secondary antibodies to the human Campath1H antibody (Figure 2). Normal peripheral blood was collected in preservative-free heparin. The leukocytes were isolated on Ficoll-Hypaque gradients and suspended at 4×10^6 cells/mL in phosphate-buffered saline. A total of 4×10^5 cells (0.1/mL) were added to 75-mm test tubes. Twenty microliters of patient serum to be tested or serial dilutions of anti-CD52 antibody diluted in normal serum to a final concentration of 100, μg/mL, 10 μg/mL, and 1 μg/mL to establish a standard curve were added to the test tubes. After incubation at 4°C for 30 minutes, the leukocytes were washed and incubated with FITC-labeled antihuman κ and antihuman λ light chains (Beckman-Coulter, Fullerton, CA) and PE-labeled murine antihuman CD3 antibody (Bec-

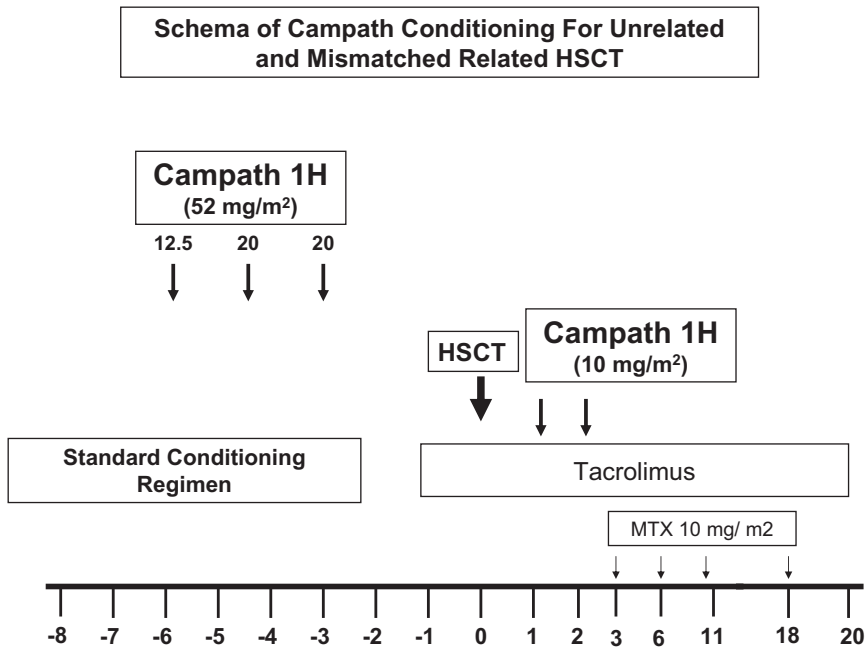

Figure 1. Schematic diagram of the protocol for Campath 1H administration to patients receiving a matched-unrelated or mismatched-related transplant. The total dosage of Campath 1H given pre-HSCT was 52 mg/m^2. The total dosage of Campath 1H given post-HSCT was 20 mg/m^2.

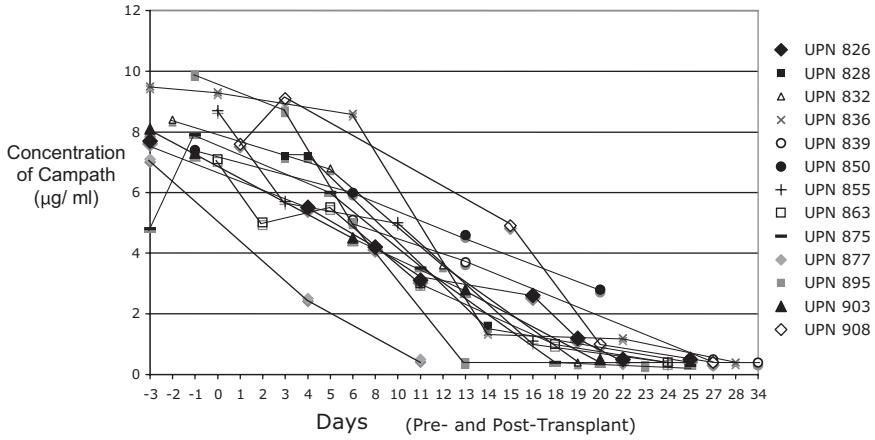

Figure 2. Levels of Campath 1H in the serum. The X-axis shows the days post-HSCT and the Y-axis the concentration of Campath 1H measured using the bioassay in μg/mL. None of the patients had detectable Campath 1H after 30 days post-HSCT.

ton-Dickinson, Mountain View, CA). After incubation at 4°C for 30 minutes, the cells were washed and fixed. The mean channel fluorescence values for FITC of the CD3-positive cells (PE-positive) stained with the serial dilutions of Campath 1H were determined and a standard curve was generated; the cells stained using patient's sera were assessed for mean fluorescence for FITC of the $CD3^+$ cells, which were read against the standard curve. The lower limit of detection of free antibody was 1 μg/mL.

Infection Prophylaxis

All patients were housed in the bone marrow transplant unit with high-efficiency particulate air filtered rooms or laminar air flow. All patients received prophylactic antibiotic, antiviral, and antifungal therapy according to our institutional standard of care. Infectious disease prophylaxis was the same for both the ATG and Campath 1H patients. Prophylaxis with ganciclovir or foscarnet was given pre-HSCT and after engraftment to all patients who were cytomegalovirus (CMV) seropositive or whose donors were CMV seropositive. All patients were tested weekly while in the hospital for virus, fungi, and bacteria. Patients had cultures or polymerase chain reaction (PCR) assays of their blood, urine, and stool for CMV and adenovirus according to our standard operating procedures. Patients who developed symptoms of other viruses were tested for these by culture or PCR if available. All patients received intravenous immunoglobulin therapy every 2 weeks while inpatients and every 4 weeks as outpatients.

Immune Reconstitution

Cellular immunologic functions were determined following HSCT. Patient immune function was evaluated by determining the absolute numbers of T, B, and NK cells, phytohemagglutinin (PHA) responses and antigen-specific T-lymphocyte blastogenesis following specific antigenic stimulation (tetanus toxoid, Candida, CMV, herpes simplex virus, and varicella zoster virus) as previously described [12].

The presence of T cells was noted when a minimum of 30 $CD3^+$ T cells/mL were detected in the peripheral blood. A PHA response was considered to be positive when there was ≥75,000 cpm ^{3}H-thymidine incorporation. Positive antigen responses were defined as a Δ cpm ≥3000. Immune functions were performed weekly after patients engrafted until the date of discharge. After patients were discharged home, immune functions were performed every 3 months.

Statistical Analysis

Results were analyzed as of November 25, 2006. The primary endpoints of this study were the presence of aGVHD and immune reconstitution. Secondary endpoints were infectious complications, relapse, and overall survival.

Fisher's exact test was used to compare categoric variables between groups (GVHD rates, infectious complications, relapse, and immune reconstitution). Group comparisons were 2-sided with a type I error of <0.05. Because of the small sample sizes, we did not have enough power to detect small to moderate differences between the 2 groups. Event-free and overall survival, as well as time to T cell reconstitution, time to PHA response, and time to antigen-specific responses were estimated using the method of Kaplan and Meier [13]. Estimates for each group are reported along with 95% confidence intervals. Breslow-Gehan-Wilcoxon tests were used to compare these time-related measures between groups.

RESULTS

Between May 2004 and May 2006, there were 14 patients who received Campath 1H as part of their

Table 3. *Median Time to the Development of Specific Immune Response (95% Confidence Intervals)*

	Campath 1H (n = 14)	ATG (n = 13)	*P* Value
Presence of ≥30 $CD3^+$ T cells/mm^3	64.5 days (42, 100) (n = 13)	27 days (23, 48) (n = 13)	.0014
Presence of normal PHA response (>75,000 cpm)	283 days (240, 420) (n = 7)	88 days (52, 120) (n = 11)	.0001
Presence of antigen-specific responses (Δ 3000 cpm)	365 days (365, 420) (n = 6)	150 days (90, 180) (n = 11)	.0037

Cpm indicates counts per minute; ATG, antithymocyte globulin.

conditioning regimen and subsequently received a MUD or mismatched-related HSCT. These patients were compared to a historic group of similar patients who received ATG (n = 13) as part of their conditioning regimen. Characteristics of the patients (Table 1) and their donors (Table 2) as well as their conditioning regimens were similar. The dose of total nucleated cells (TNC) infused was similar between the 2 groups. The 2 groups were then compared in terms of the presence of severe aGVHD, infectious complications, immune reconstitution, relapse, and survival.

Campath 1H Levels

Campath 1H levels were determined on the first 13 patients of this study (Figure 2). The 13 patients cleared Campath 1H at a median of 20 days post-HSCT (range: 11-28 days). No detectable (<1 μg/mL) Campath1H was seen in any of the remaining patients after day 30 post-HSCT. The half-life of Campath 1H was determined to be 5.36 days (2.4-6.3 days), with a standard deviation of 1.2 days.

GVHD

GVHD was evaluated in all recipients of unrelated HSCT. Severe aGVHD (defined as grade III or IV according to the Glucksberg scale) [11] was seen in 0 of 14 patients receiving Campath 1H compared to 6 of 13 patients who received ATG ($P = .006$). Two recipients of Campath 1H developed mild (Grade 1) skin GVHD, which was controlled with low-dose corticosteroids. One of the 2 Campath patients who developed GVHD relapsed his disease (CML) 2 years post-HSCT. Of the 12 patients who did not develop any GVHD, 1 patient died of relapse of his primary leukemia.

Of the 6 patients who received ATG and developed severe GVHD, all were subsequently treated with a combination of a calcineurin inhibitor, high-dose steroids, and Daclizumab or other agents for prolonged duration to control their GVHD. Five recipients of ATG developed Grade 1-2 GVHD, which was controlled with corticosteroids alone. Of the remaining 2 patients who did not develop any GVHD, 1 patient died of relapse of his primary leukemia.

Of the 14 patients who received Campath 1H, 8 patients are surviving. Of these patients, 1 patient has extensive chronic GVHD (cGVHD). This patient was noncompliant with her medications. Her GVHD is now under control with 3 immunosuppressive medications. Of the 13 patients who received ATG, 8 patients are surviving; 5 have extensive cGVHD and remain on immunosuppression.

Immune Reconstitution

All patients were evaluated weekly after engraftment for the development of T cells. Immune reconstitution for patients receiving Campath 1H and ATG are shown in Table 3. T cell reconstitution was defined as the first time point that patients had an absolute number of ≥30 $CD3^+$ T cells/mm^3 in the peripheral blood. Median time to T cell reconstitution in the peripheral blood occurred later in the Campath 1H group compared to the ATG group. For patients receiving Campath 1H, T cell reconstitution in the peripheral blood occurred at a median of 64.5 days post-HSCT (range: 33-140 days, n = 13). Patients who received ATG had T cell reconstitution in the peripheral blood at a median of 27 days post-HSCT (18-68 days, n = 13). The difference between the times to T cell reconstitution was statistically different (Campath 1H; 64.5 days versus ATG: 27 days, $P = .0014$).

Median time to the development of normal PHA responses (≥75, 000 cpm) occurred later in the Campath 1H group compared to those who received ATG. For patients receiving Campath 1H, normal PHA responses occurred at a median of 283 days (range: 148-420 days, n = 7). Five patients in the Campath 1H group died prior to the development of PHA responses. Two patients have not developed PHA responses at days 193 and 244 post-HSCT. Patients who received ATG had normal PHA responses at a median of 90 days post-HSCT (range: 46-216 days, n = 11). Two patients died prior to the development of PHA responses in the ATG group. The difference between the times to normal PHA responses was statistically different (Campath 1H, 283 days versus ATG, 90 days, $P = .0001$).

Median time to the development of antigen-specific responses also was later in the Campath 1H group compared to the ATG group. Recipients of Campath 1H developed antigen specific responses at a median of 365 days (range: 225-425 days, n = 6). Prior to developing antigen specific responses in the Cam-

Table 4. *Fungal and Viral Infections Among Patients*

	ATG (n = 13)	Campath 1H (n = 14)	*P* Value
Adenovirus	6	5	.77
BK virus	0	4	.1
CMV	1	4	.33
Candida (blood/urine/stool)	6 2/1/3	2 0/0/2	.1
EBV	1	0	.48
HHV6	0	3	.22
HSV	2	0	.22
Influenza	1	0	.48
Parainfluenza	0	1	1.00
Rotavirus	0	1	1.00
RSV	1	0	.48
Toxoplasmosis	0	1	1.00
Total infections	18	21	

ATG indicates antithymocyte globulin; HSV, herpes simplex virus; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HSV, herpes simplex virus; RSV, respiratory syncytial virus.

path 1H group, 5 patients died, 1 patient relapsed, and 3 patients are still awaiting antigen-specific responses (days 193, 244, and 492 post-HSCT). Patients who received ATG had antigen-specific responses at a median of 150 days post-HSCT (range: 88-840 days, n = 11). Prior to development of antigen-specific responses in the ATG group, 2 patients died. The difference between the times to antigen-specific responses was statistically different (Campath 1H, 365 days, versus ATG, 150 days, $P = .004$).

Infectious Complications

All patients were analyzed for the development of viral and fungal disease for 1-year post-HSCT. All patients had quantitative testing for CMV weekly. Other viruses were tested quantitatively if symptoms were present. Human herpes virus (HHV)-6 was not tested routinely prior to 2005. Table 4 shows the organisms that were isolated. There were no significant differences between Campath 1H and ATG for the numbers of organisms isolated (Campath 1H, 21 total infections versus ATG, 18 total infections). It should be noted that there was not enough power to detect small to moderate differences between the 2 groups. However, when examining the patients who developed specific infections, there were higher absolute numbers of patients who developed CMV, HHV6, or BK virus among the patients who received Campath 1H compared to those who received ATG (4 CMV, 3 HHV6, 4 BK virus [Campath 1H] versus 1 CMV, 0 HHV6, and 0 BK virus [ATG]). The patients who developed HHV6 had symptoms of HHV6 infection (pulmonary symptoms [n = 1] and bone marrow suppression [n = 2]). Three of the 4 patients who had BK virus had the presence of hemorrhagic cystitis. Patients who received ATG had more absolute numbers of candidal infections (Campath 1H [n = 2] versus ATG [n = 6], $P = .1$) compared to the patients who received Campath 1H. All patients received fluconazole prophylaxis, unless they were treated for presumed fungal infection prior to HSCT with alternate antifungal infections. There was 1 death resulting from infection in each group: Campath 1H (1 Toxoplasmosis-related death) versus ATG (1 Epstein-Barr virus [EBV]-related death).

Engraftment and Relapse

All patients in both the Campath 1H group and the ATG group engrafted. In the Campath 1H group, 1 patient has mixed chimerism (65% donor engrafted) and requires periodic blood and platelet transfusions.

Patients with a malignant disease were also evaluated for relapse. Of the 14 patients with leukemia who received Campath 1H, 2 patients relapsed (14%); of the 9 patients with leukemia who received ATG, 4 patients relapsed (44%) ($P = .16$). Of the 2 patients who received Campath 1H and relapsed, 1 died of progressive disease and the other is alive. All patients who relapsed post-HSCT in the ATG group have died of progressive disease.

Survival

Kaplan-Meier overall and event-free survival curves are shown in Figure 3A and B. The median follow-up for patients receiving Campath 1H is 674 days (range: 193-921 days). The median follow up for patients receiving ATG is 1656 days (range: 818-2390 days).

All patients were observed for at least 180 days. The Kaplan-Meier curves were comparable for overall survival (Wilcoxon $P = .36$) and event-free survival (Wilcoxon $P = .4$). The event-free survival for patients receiving Campath 1H at 100 days, 180 days, and 1 year was 79%, 71%, and 63%. The event-free survival for patients receiving ATG at 100 days, 180 days, and 1 year was 85%, 77%, and 69%.

The overall survival at 100 days, 180 days, and 1 year for patients receiving Campath 1H was 79%, 71%, and 63%. The overall survival at 100 days, 180 days, and 1 year for patients receiving ATG was 92%, 85%, and 69%. At 100 days, the proportion still alive did not differ significantly between the groups (79% for Campath versus 92% for ATG, Fisher's exact test $P = .60$), nor did the event-free proportion (79% for Campath versus 85% for ATG, Fisher's exact test $P = 1.00$).

Causes of death in the Campath 1H group included: relapse (n = 1), toxoplasmosis (n = 1), leukoencephalopathy (n = 1), VOD (n = 1), intracranial hemorrhage (n = 1), and cardiac failure (n = 1). Causes of death in the ATG group in-

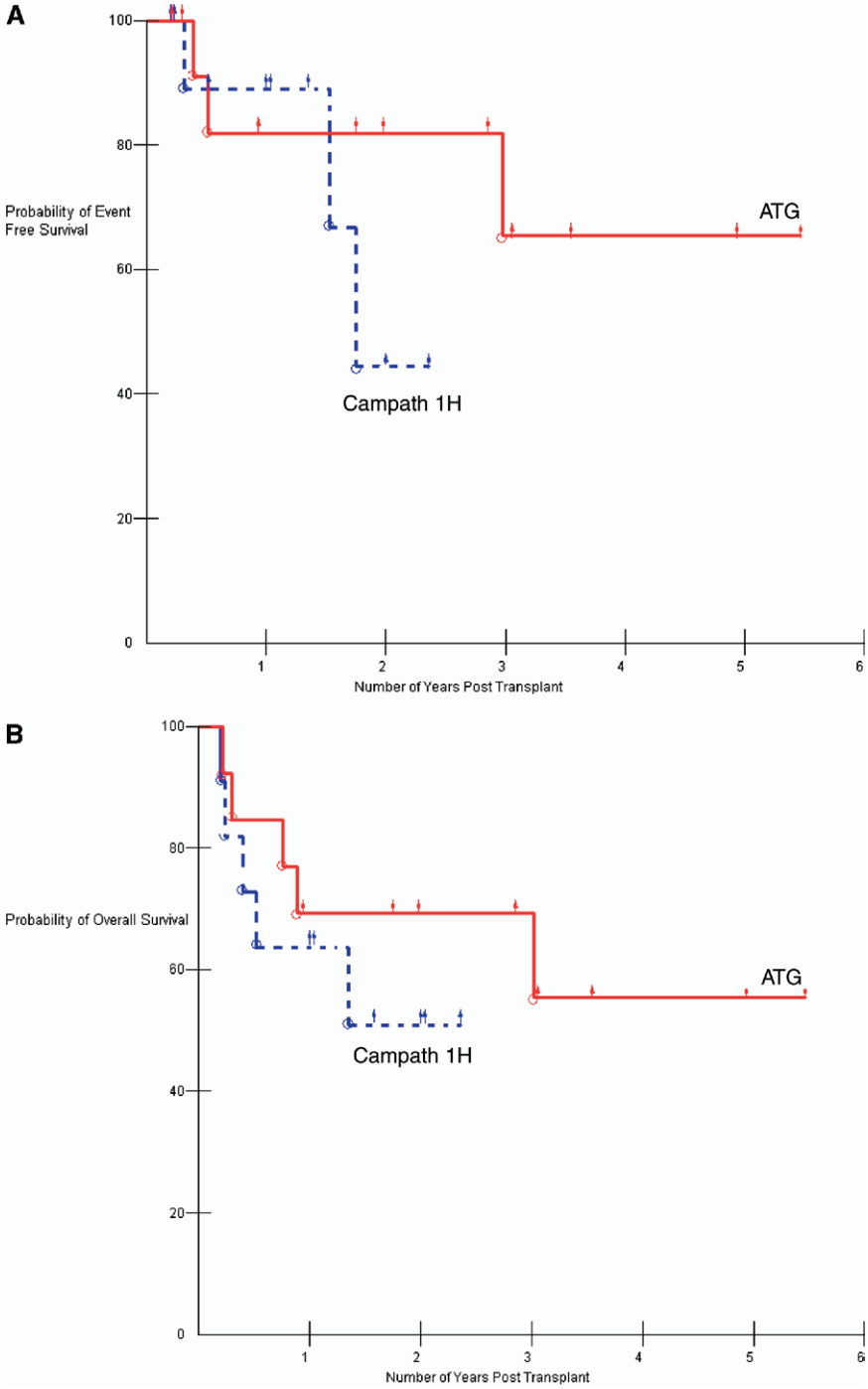

Figure 3. (A) Event-free survival of patients in the Campath 1H versus ATG groups. Patients were censored for death, relapse, or rejection. There was no significant difference in the event-free survival between the 2 groups (Wilcoxon *P*-value = .40). (B) Overall survival of patients in the Campath 1H versus ATG groups. There was no significant difference in the overall survival between the 2 groups (Wilcoxon *P*-value = .36).

cluded: relapse (n = 4) and EBV-related lymphoproliferative disease (n = 1).

DISCUSSION

Despite prophylactic measures, GVHD remains a significant cause of morbidity and mortality among recipients of matched-unrelated and mismatched-related HSCT. Once GVHD occurs, the treatment remains unsatisfactory. Corticosteroids have been a primary treatment for aGVHD [14]. Treatment with corticosteroids have given a durable response in <50% of patients receiving unrelated HSCT [15,16]. For patients with GVHD for whom steroids fail, there

are a variety of new agents that have been used, with less than optimal results. Although these newer therapies (eg, daclizumab [17], denileukin diftitox [18], and pentostatin [19]) have treated GVHD, there were often increased opportunistic infections that resulted in mortality.

The purpose of this pilot pediatric study was to determine if Campath 1H was able to decrease the incidence of GHVD without increasing infections or relapse. With the use of Campath 1H, the incidence of severe GVHD was significantly lower compared to those who received our previous regimen using ATG. In addition our patients received 2 doses (10 mg/m^2) on days 1 and 2. Using a bioassay to detect Campath 1H in the patient serum, we were able to detect the presence of circulating Campath 1H at the time of transplantation and remaining until 30 days post-HSCT. This may have resulted in a degree of in vivo T cell depletion that was able to minimize extensive aGVHD. Ongoing evaluations will determine whether there will be any difference in cGVHD compared to our historic controls.

Previous studies have shown that T cell-depleted transplants result in higher relapse rates [20]. In our study, we did not observe an increase in relapse. However, this statement should be taken with caution, because the follow-up on the Campath 1H patients was lower than that of the patients receiving ATG. Longer follow-up is needed before we can ascertain whether Campath 1H does not have an impact on relapse.

Compared to the patients receiving ATG, there was a significant delay in immune reconstitution in patients who received Campath 1H. This is similar to the adult trials where median time to T cell recovery was also delayed [8,9]. To our knowledge, our study is the first to evaluate the immune reconstitution in a pediatric only population. The prolonged immunosuppression seen in patients receiving Campath 1H did not result in an increase in viral or fungal infections compared to recipients of ATG. This is in contrast with other studies that showed in increase in infections, especially CMV and adenovirus [7-10]. CMV has been a known complication among patients receiving Campath 1H [8,9]. At our institution, testing for CMV was the same from 2002-2006, spanning the times for both the ATG and the Campath 1H groups. One of the major differences between our study and that of others is the aggressive anti-CMV prophylaxis used at our institution. At CHLA, patients who are CMV seropositive or have a donor who is CMV seropositive are given gancyclovir pre- and post-HSCT. In previous studies, that showed an increase in CMV infections, patients were given antiviral therapy only if 2 consecutive qualitative CMV PCR assays were positive [9,10]. Recent reports have shown that pre- and posttransplant gancylcovir reduces the incidence of CMV infections after Campath 1H-based conditioning [21]. Our study is in support of the more aggressive use of prophylaxis to decrease the incidence of CMV infections in Campath 1H-based conditioning regimens.

Our study did show an increase in the absolute numbers of Campath 1H recipients developing certain viral infections (CMV, HHV6, and BK virus). One possible explanation for the increase in HHV6 and BK virus may be the more recent increase in routine testing for these specific viruses. Testing for HHV6 became more frequent at our institution after 2005. Because it is known that the anti-CD3 monoclonal antibody treatment administered as a prophylaxis for GVHD also increases the risk of HHV6 infections [22], we began more frequent testing for HHV6 after using Campath 1H in our conditioning regimen. Approximately 40% of HSCT recipients develop an HHV6 infection within 2-4 weeks post-HSCT [23]. Therefore, we do not know if the increase in absolute numbers of HHV6 infections represented a true increase in the numbers of patients or if this is increase resulted from more frequent testing for this virus.

BK viruria occurs in nearly all HSCT recipients but has a low predictive value for the development of BK viremia [24-26]. In general, the incidence of BK virus in recipients of pediatric HSCT recipients is unknown. We began routinely testing for BK virus in 2004. Of the patients who were found to have BK virus, 3 had hematuria and 1 was asymptomatic. Because patients receiving the ATG regimen were not tested as often for polyoma virus, it is unclear if there were previous patients who also had polyoma virus but were asymptomatic.

The numbers of infection-related deaths in our study were not significantly different between the patients given ATG or Campath 1H. Possible explanation for this observation is that patients given Campath 1H did not develop GVHD following viral disease. It is possible that the infections in the Campath 1H recipients did not trigger an increase in GVHD because of the patient's relative T cell deficiency, thus allowing for the effective treatment of infections in the Campath 1H group without the need to simultaneously increase the intensity of immune suppression.

One of the limitations of this study is the small numbers of pediatric patients compared to most larger adult studies. Larger pediatric cooperative group trials using Campath 1H for unrelated HSCT, will provide more information about its potential efficacy in decreasing GVHD without increasing infections or relapse. A second limitation of this study is the shorter follow-up seen in the patients with Campath 1H compared to our historic control. Longer follow-up is needed to assess the role of Campath 1H in pediatric HSCT.

In this pilot study, the recipients of Campath 1H and ATG had similar outcomes in terms of infections, relapse, and overall survival. Recipients of Campath 1H did have a significant delay in immune reconstitution. CD52 is present on the common lymphoid progenitor (G. Crooks, unpublished observation) and Campath 1H may decrease the numbers of this progenitor population that engraft and proliferate to effect early immune reconstitution.

In conclusion, Campath 1H is an effective means to decrease the incidence of aGVHD. Campath 1H has changed the clinical course of our patients undergoing unrelated donor and mismatched-related HSCT by completely eliminating serious aGVHD. With proactive use of monitoring and prophylactic antivirals, antifungals, and antibacterials, the numbers of viral or fungal infections were not significantly increased in our study group compared to our historic control. Because of the observation of delayed immune reconstitution after Campath 1H, further studies will be aimed at decreasing the dosages of Campath1H without increasing the incidence of GVHD.

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